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SECOND GENUS CROSSED ORBITS[1]

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Abstract

In a former paper, first genus periodic orbits were obtained for the motion of the electrons in the "crossed orbit" model of the normal helium atom as proposed by Kemble and Bohr. The present paper treats the second genus orbits, i.e., orbits which are in the vicinity of the first genus orbits and which re-enter after the lapse of many first genus periods.

The methods employed involve the solutions of differential equations with periodic coefficients. Notation is introduced which greatly expedites the obtaining of these solutions.

1. Introduction

The problem considered in this paper is to determine second genus periodic orbits for the motion of two infinitesimal bodies that repel each other and are attracted by a third finite body. The forces of attraction and repulsion vary according to the Newtonian law of the inverse square.

As the three bodies in this problem are somewhat analogous to the normal helium atom, the finite body will be referred to as the nucleus and the infinitesimal bodies as the electrons.

2. First Genus Orbits

One periodic orbit is that in which the electrons move in a circle with the nucleus as centre, the electrons remaining diametrically opposite. This plane of motion will be called the *equatorial plane*. Angular distance on either side of it will be called *latitude*, while *longitude* will have the obvious meaning. In a previous paper (2) the author obtained two types of three-dimensional periodic oscillations in the vicinity of this circular orbit. In the first case the longitudes differ by 180°, whereas the latitudes are equal in magnitude but opposite in sign. In the second case the longitudes differ by 180°, but the latitudes are equal in magnitude and also in sign.

The results that were obtained in the paper just cited are similar to those that were first proposed by Kemble and later independently by Bohr for the "Crossed Orbit" model of the normal helium atom (6, Chap. VII).

The present paper treats the second genus orbits for Case I of the author's previous paper cited.

[1] *Manuscript received September 13, 1941.*

Contribution from the Faculty of Arts and Science, The University of British Columbia, Vancouver, B.C. Read at the May, 1940, meeting of the Royal Society of Canada.

[2] *Dean.*

3. The Differential Equations

Choose a system of rectangular axes with the nucleus as origin and the equatorial plane as the xy-plane. Let the ratio of repulsion to attraction be denoted by k^2 and let the units of time and of distance be so chosen that the gravitational constant of attraction is unity. If the co-ordinates of the electrons are x_1, y_1, z_1, and x_2, y_2, z_2, the force function is

$$U = \frac{1}{r_1} + \frac{1}{r_2} - \frac{k^2}{r_{12}},$$

$$r_1 = (x_1^2 + y_1^2 + z_1^2)^{\frac{1}{2}}, \qquad r_2 = (x_2^2 + y_2^2 + z_2^2)^{\frac{1}{2}},$$

$$r_{12} = [(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2]^{\frac{1}{2}},$$

and the differential equations of motion are

$$\frac{d^2x_j}{dt^2} = \frac{\partial U}{\partial x_j}, \quad \frac{d^2y_j}{dt^2} = \frac{\partial U}{\partial y_j}, \quad \frac{d^2z_j}{dt^2} = \frac{\partial U}{\partial z_j}, \tag{1}$$

$$j = 1, 2.$$

In Case I of the paper cited above

$$x_2 = -x_1, \quad y_2 = -y_1, \quad z_2 = -z_1. \tag{2}$$

Then $r_1 = r_2 = \frac{1}{2}r_{12}$. It is then necessary to consider the motion of only one electron, say x_1, y_1, z_1, and the differential equations of its motion are

$$\frac{d^2x_1}{dt^2} = -\frac{\mu x_1}{r_1^3}, \quad \frac{d^2y_1}{dt^2} = -\frac{\mu y_1}{r_1^3}, \quad \frac{d^2z_1}{dt^2} = -\frac{\mu z_1}{r_1^3}, \tag{3}$$

where $\mu = 1 - \frac{1}{4}k^2$ and $0 < \mu < 1$. Transforming to cylindrical co-ordinates r, θ, z and eliminating $\frac{d\theta}{dt}$ by use of the integral of areas $r^2\frac{d\theta}{dt} = c$, a constant, we obtain the equations

$$\left.\begin{aligned} \frac{d^2r}{dt^2} &= \frac{c^2}{r^3} - \frac{\mu r}{(r^2 + z^2)^{3/2}}, \\ \frac{d^2z}{dt^2} &= -\frac{\mu z}{(r^2 + z^2)^{3/2}}. \end{aligned}\right\} \tag{4}$$

4. The First Genus Orbits

A particular solution of Equations (4) is

$$r = c^2/\mu, \qquad z = 0,$$

being the case in which the electrons move in a circle in the equatorial plane and remain diametrically opposite. To obtain the differential equations of displacement put

$$\left.\begin{aligned} r &= \frac{c^2}{\mu}(1 + \epsilon\rho), \qquad z = \frac{c^2}{\mu}(\epsilon\zeta), \\ t - t_0 &= \frac{c^3}{\mu^2}(1 + \delta)^{\frac{1}{2}}\tau, \end{aligned}\right\} \tag{5}$$

where ρ, ζ are new dependent variables, τ is the new independent variable, and δ and ϵ are parameters. Thus

$$\begin{aligned}\ddot{\rho} + (1+\delta)\rho &= R = (1+\delta)\Big[\epsilon(3\rho^2 + \frac{3}{2}\zeta^2) - 6\epsilon^2(\rho^3 + \rho\zeta^2) \\ &\quad + \epsilon^3(10\rho^4 + 15\rho^2\zeta^2 - \frac{15}{8}\zeta^4) + - - -\Big], \\ \ddot{\zeta} + (1+\delta)\zeta &= Z = (1+\delta)\Big[3\epsilon\rho\zeta - \epsilon^2(6\rho^2\zeta - \frac{3}{2}\zeta^3) + \epsilon^3(10\rho^2\zeta - \frac{15}{2}\rho\zeta^3) \\ &\quad + \epsilon^4\Big(-15\rho^4\zeta + \frac{45}{2}\rho^2\zeta^3 - \frac{15}{8}\zeta^5\Big) + - - -\Big],\end{aligned} \tag{6}$$

where the dots denote derivation with respect to τ. The right member R is even in ζ and Z is odd in ζ. In the coefficients of the various powers of ϵ it is found that in R the parity of ρ is opposite that of ϵ, and in Z it is the same as that of ϵ. From these properties it is seen that ρ is odd in ϵ while ζ and δ are both even in ϵ. If the initial values

$$\dot{\rho}(0) = \zeta(0) = 0, \qquad \dot{\zeta}(0) = 1, \tag{7}$$

are taken, the periodic solutions of Equations (6) are

$$\left.\begin{aligned}\rho &= \frac{1}{4}(3 + \cos 2\tau)\epsilon - \frac{1}{64}(51 + 12\cos 2\tau + \cos 4\tau)\epsilon^3 + - -, \\ \zeta &= \sin\tau + (0)\epsilon^2 + (0)\epsilon^4 + (\ )\epsilon^6 + - -, \\ \delta &= 3\epsilon^2 + (0)\epsilon^4 + (\ )\epsilon^6 + - -.\end{aligned}\right\} \tag{8}$$

When these results are substituted in Equations (5) the equations for the periodic orbits of the first genus are obtained.

SECOND GENUS ORBITS

5. Definition

Given a set of differential equations

$$\frac{dx_i}{dt} = X_i(x_j, \epsilon; t), \qquad i, j = 1, - - -, n,$$

where the X_i are analytic in the arguments x_j, ϵ and do not contain t explicitly. Suppose they admit the periodic solutions

$$x_i = \theta_i(\epsilon, t)$$

having the period T. These solutions are said to be of the *first genus*. If we substitute

$$\epsilon = \epsilon_0(1 + \lambda), \qquad x_i = \theta_i(\epsilon_0, t) + y_i$$

in the differential equations then solutions for the y_i, say

$$y_i = \Psi_i(\epsilon_0, \lambda; t),$$

can be found having the period

$$NT(1 + \text{a power series in } \lambda),$$

N being an integer. The solutions

$$x_i = \theta_i(\epsilon_0, t) + \Psi_i(\epsilon_0, \lambda; t)$$

are said to be of the *second genus* (5, Vol. 3, Chap. XXVIII). The second genus orbits approach those of the first genus as the parameter λ approaches zero.

6. The Differential Equations

Let $\epsilon = \epsilon_0(1 + \lambda)$, where ϵ occurs explicitly in Equations (6) but not where it enters implicitly through ρ, ζ, and δ. Let ρ_0, ζ_0, δ_0 represent the values in Equations (8) when ϵ is replaced by ϵ_0. Further, let

$$\rho = \rho_0 + p, \qquad \zeta = \zeta_0 + q \tag{9}$$

and let $(1 + \delta)$ be replaced by $(1 + \delta)(1 + \gamma)$ where p, q are dependent variables and γ is a parameter that vanishes with λ. The above substitutions will be made in Equations (6) and for the time being the subscript $_0$ on ϵ, δ, ρ, ζ will be dropped. Hence Equations (6) become

$$\begin{aligned}
&\ddot{\rho} + \ddot{p} + (1 + \delta)(1 + \gamma)(\rho + p) = (1 + \delta)(1 + \gamma)\Big[3\epsilon(1 + \lambda)\{(\rho + p)^2 \\
&\quad + \tfrac{1}{2}(\zeta + q)^2\} - 6\epsilon^2(1 + \lambda)^2\{(\rho + p)^3 + (\rho + p)(\zeta + q)^2\} + - - -\Big], \\
&\ddot{\zeta} + \ddot{q} + (1 + \delta)(1 + \gamma)(\zeta + q) = (1 + \delta)(1 + \gamma)\Big[3\epsilon(1 + \lambda)(\rho + p) \\
&\quad (\zeta + q) - 3\epsilon^2(1 + \lambda)^2\{2(\rho + p)^2(\zeta + q) - \tfrac{1}{2}(\zeta + q)^3\} + - - -\Big]
\end{aligned} \tag{10}$$

where the terms independent of p, q, γ, λ cancel off. When the solutions for ρ, ζ are substituted in the above we obtain

$$\begin{aligned}
\ddot{p} + P_{11}p + P_{12}q &= P_1, \\
\ddot{q} + Q_{11}p + Q_{12}q &= Q_1,
\end{aligned} \tag{11}$$

where

$$P_{11} = 1 + \frac{3}{2}\epsilon^2(1 - 3\cos 2\tau) + \frac{3}{32}\epsilon^4(17 + 20\cos 2\tau + 27\cos 4\tau) + - - -,$$

$$P_{12} = Q_{11} = -3\epsilon\sin\tau + \frac{3}{8}\epsilon^3(11\sin\tau - \sin 3\tau) + - -,$$

$$Q_{12} = 1 - \frac{3}{2}\epsilon^2(1 - \cos 2\tau) + 3\epsilon^4(1 - \cos 2\tau) + - -.$$

The values for P_1, Q_1 will be determined later.

7. The Equations of Variation

If the right members of Equations (11) are neglected,

$$\ddot{p} + P_{11}p + P_{12}q = 0, \qquad \ddot{q} + Q_{11}p + Q_{12}q = 0, \tag{12}$$

and these are called the *equations of variation*. Their *generating solutions* are

$$\begin{aligned}
\epsilon\rho &= \frac{1}{4}\epsilon^2(3 + \cos 2\tau) - \frac{1}{64}\epsilon^4(51 + 12\cos 2\tau + \cos 4\tau) + - - -, \\
\epsilon\zeta &= \epsilon\sin\tau + (\ )\epsilon^6 + - - -.
\end{aligned} \tag{13}$$

To obtain the solutions of Equations (12) the usual substitutions

$$p = e^{i\alpha\tau}u, \qquad q = e^{i\alpha\tau}v, \tag{14}$$

are made, where α is a constant and u and v are functions of τ. Equations (12) then become

$$\left.\begin{aligned}
(D^2 + 2i\alpha D - \alpha^2 + P_{11})u + P_{12}v &= 0, \\
Q_{11}u + (D^2 + 2i\alpha D - \alpha^2 + Q_{12})v &= 0,
\end{aligned}\right\} \tag{15}$$

$$D \equiv \frac{d}{d\tau}.$$

The existence proof, which is omitted here, shows that α, as a power series in ϵ^2, and certain initial values can be determined so that u and v will be periodic with the period 2π.

Now P_{12} and Q_{11} are odd in ϵ while P_{11}, Q_{12} and α are even in ϵ. Then u and v are of opposite parity in ϵ. Since Solutions (14) are later multiplied by arbitrary constants we may for one set of solutions select u as even and v as odd or u as odd and v as even. The former selection will be made and accordingly we assume

$$\left.\begin{aligned} u &= u^{(0)} + u^{(2)}\,\epsilon^2 + u^{(4)}\,\epsilon^4 + - - -, \\ v &= v^{(1)}\,\epsilon + v^{(3)}\,\epsilon^3 + - - -, \\ \alpha &= 1 + \alpha_2\,\epsilon^2 + \alpha_4\,\epsilon^4 + - - -. \end{aligned}\right\} \tag{16}$$

Let Equations (16) be substituted in Equations (15) and let the resulting equations be denoted as (15′). We then equate to zero the coefficients of the various powers of ϵ in (15′) and we thus obtain sets of differential equations that define the various $u^{(2j)}$ and $v^{(2j+1)}$. It will now be shown that the various α_{2j} and certain constants of integration can be determined so that each $u^{(2j)}$ and $v^{(2j+1)}$ will be periodic.

The question of the initial values for u and v will be deferred until after the first step of the integration.

Step 1. Terms in (15′) Independent of ϵ

There is but one differential equation at each step. At this step it is

$$(D^2 + 2iD)u^{(0)} = 0,$$

and its solution is

$$u^{(0)} = A_0 + B_0 e^{-2i\tau}.$$

There are two arbitrary constants A_0 and B_0. If we assign the initial value $u(0) = 1$, the solution would be $u^{(0)} = A_0 + (1 - A_0)e^{-2i\tau}$. The integration was carried out with this initial value and the u's and v's were found to contain exponentials having the highest multiples of τ exceeding by two the power of ϵ associated with the u's and v's. This leads to a somewhat complicated form for the final solutions. We therefore choose the initial value $\dot{u}(0) = 0$ from which it follows that each $\dot{u}^{(2j)}(0) = 0$. The desired solution at the first step is then $u^{(0)} = A_0$, where A_0 remains arbitrary.

Step 2. Terms in ϵ

The differential equation is

$$(D^2 + 2iD)v^{(1)} = (3 \sin \tau)u^{(0)},$$

and the solution expressed in terms of exponentials is

$$v^{(1)} = C_1 + D_1 e^{-2i\tau} + \frac{iA_0}{2}(e^{i\tau} + 3e^{-i\tau}),$$

where A_0, C_1, D_1 are arbitrary.

Step 3. Terms in ϵ^2

The differential equation is

$$(D^2 + 2iD)u^{(2)} = U^{(2)} = \left(2\alpha_2 - \frac{3}{2} + \frac{9}{2}\cos 2\tau\right)u^{(0)} + (3 \sin \tau)v^{(1)}.$$

Non-periodic terms will arise from the constant terms and terms in $e^{-2i\tau}$ in $U^{(2)}$. These terms are

$$U^{(2)} = 2A_0\alpha_2 + (0)e^{-2i\tau}.$$

Hence either A_0 or α_2 must be zero. To put $A_0 = 0$ would lead to the trivial solution, hence we must put $\alpha_2 = 0$. The integration then yields

$$u^{(2)} = A_2 - \frac{3}{8}A_0(e^{2i\tau} + e^{-2i\tau}) + \frac{i}{2}[C_1e^{i\tau} + 3(C_1 - D_1)e^{-i\tau} + D_1e^{-3i\tau}], \quad (17)$$

where A_0, A_2, C_1, D_1 are arbitrary.

Step 4. Terms in ϵ^3

The differential equation is

$$(D^2 + 2iD)v^{(3)} = V^{(3)} = -\frac{3}{8}(11\sin\tau - \sin 3\tau)u^{(0)} + (3\sin\tau)u^{(2)} + \frac{3}{2}(1 - \cos 2\tau)v^{(1)}.$$

Now $V^{(3)}$ contains the terms

$$V^{(3)} = 3\left(C_1 - D_1\right) - \left(3C_1 - \frac{11}{2}D_1\right)e^{-2i\tau}, \quad (18)$$

and these terms must be annulled if $v^{(3)}$ is to be periodic. Hence $C_1 = D_1 = 0$ and $u^{(2)} = A_2 - \frac{3}{8}A_0(e^{2i\tau} + e^{-2i\tau})$. The integration for $v^{(3)}$ will then be

$$v^{(3)} = C_3 + D_3e^{-2i\tau} + \frac{iA_2}{2}(e^{i\tau} + 3e^{-i\tau}) - \frac{iA_0}{8}(3e^{i\tau} - 3e^{-i\tau} - 4e^{-3i\tau}),$$

where A_0, A_2, C_3, D_3 are arbitrary. The constants C_3, D_3 remain arbitrary through Step 5 but at Step 6 they enter $V^{(5)}$ the same as C_1, D_1 occur in Equation (18). Hence $C_3 = D_3 = 0$ and these terms will be ignored in $v^{(3)}$ above.

Step 5. Terms in ϵ^4

The differential equation is

$$(D^2 + 2iD)u^{(4)} = U^{(4)} = 2\alpha_4u^{(0)} - \frac{3}{2}(1 - \cos 2\tau)u^{(2)} - \frac{3}{32}(17 + 20\cos 2\tau + 27\cos 4\tau)u^{(0)} + (3\sin\tau)v^{(3)} - \frac{3}{8}(11\sin\tau - \sin 3\tau)v^{(1)}. \quad (19)$$

So far as the constants and terms in $e^{-2i\tau}$ enter $U^{(4)}$ we have

$$U^{(4)} = A_0\left(2\alpha_4 - \frac{99}{32}\right) - \left(\frac{3}{2}A_2 - \frac{45}{16}A_0\right)e^{-2i\tau}.$$

Hence

$$\alpha_4 = -\frac{99}{64}A_0, \qquad A_2 = \frac{15}{8}A_0,$$

and the desired solution for $u^{(4)}$ is

$$u^{(4)} = A_4 - A_0\left(\frac{335}{256}e^{-2i\tau} + \frac{9}{64}e^{2i\tau} - \frac{93}{1536}e^{4i\tau} - \frac{165}{256}e^{-4i\tau}\right),$$

where A_0, A_4 are arbitrary.

The General Step

At the 7th step, $U^{(6)}$ contains the terms

$$U^{(6)} = A_0(2\alpha_6 + \text{constant}) - \left\{\frac{3}{2}A_4 + A_0\,(\text{constant})\right\}\epsilon^{-2i\tau},$$

and there are no other terms where α_6 and A_4 enter. Hence α_6 and A_4 can be uniquely determined. The arbitrary constants arising through a v-equation enter the next v-equation as in Equation (18) and must be annulled. An induction to the general term will readily show that the α's and the constants of integration can be uniquely determined as above. Since A_0 remains arbitrary we may take $A_0 = 1$, as we later multiply u by an arbitrary constant.

One set of solutions of the equations of variation has thus been found, viz.,

$$p = e^{i\alpha\tau}u_1, \qquad q = e^{i\alpha\tau}iv_1, \tag{20}$$

where

$$u_1 = 1 - \frac{3}{4}\epsilon^2(\cos 2\tau) + - - - ,$$

$$= \sum_{j=0}^{\infty}\sum_{k=0}^{j}[A_k^{(2j)}\cos 2k\tau + i\,B_k^{(2j)}\sin 2k\tau]\epsilon^{2j},$$

$$v_1 = \epsilon\,(2\cos\tau - i\sin\tau) + (\ )\epsilon^3 + - - ,$$

$$= \sum_{j=0}^{\infty}\sum_{k=0}^{j}[C_k^{(2j+1)}\cos(2k+1)\tau + i\,D_k^{(2j+1)}\sin(2k+1)\tau]\epsilon^{2j+1},$$

$$\dot{u}_1(0) = 0.$$

A second set of solutions can be obtained by changing the sign of i in Equations (20) thus

$$p = e^{-i\alpha\tau}u_2, \qquad q = e^{-i\alpha\tau}(-iv_2), \tag{21}$$

where

$$u_2 = \bar{u}_1, \qquad v_2 = \bar{v}_1.$$

The remaining two sets of the solutions of the equations of variation can be obtained, as shown by Poincairé (5, Vol. 1, Chap. IV), by partial differentiation of the generating solutions Nos. (13) with respect to the two arbitrary constants that they contain, viz., the initial time t_0 and the scale factor ϵ.

Differentiating Equations (13) first with respect to t_0 we obtain

$$p = \frac{\partial(\epsilon\rho)}{\partial t_0} = -\frac{\mu^2}{c^3}\frac{1}{(1+\delta)^{1/2}}\frac{\partial(\epsilon\rho)}{\partial\tau} = -\frac{\mu^2}{c^3}\frac{1}{(1+\delta)^{1/2}}u_3,$$

where

$$u_3 = -\frac{1}{2}(\sin 2\tau)\epsilon^2 + \frac{1}{16}(6\sin 2\tau + \sin 4\tau)\epsilon^4 + - - - ,$$

$$q = \frac{\partial(\epsilon\zeta)}{\partial t_0} = -\frac{\mu^2}{c^3}\frac{1}{(1+\delta)^{1/2}}\frac{\partial(\epsilon\zeta)}{\partial\tau} = -\frac{\mu^2}{c^3}\frac{1}{(1+\delta)^{1/2}}v_3,$$

where

$$v_3 = (\cos\tau)\epsilon + (\ )\epsilon^7 + - - .$$

Since these solutions are later multiplied by arbitrary constants, we may drop the constant multipliers of u_3 and v_3 and take

$$p = u_3, \qquad q = v_3 \tag{22}$$

as the solutions.

Differentiating Equations (13) with respect to ϵ we obtain

$$p = \left(\frac{\partial(\epsilon\rho)}{\partial\epsilon}\right) + \frac{\partial(\epsilon\rho)}{\partial\tau}\frac{\partial\tau}{\partial\delta}\frac{\partial\delta}{\partial\epsilon},$$

$$q = \left(\frac{\partial(\epsilon\zeta)}{\partial\epsilon}\right) + \frac{\partial(\epsilon\zeta)}{\partial\tau}\frac{\partial\tau}{\partial\delta}\frac{\partial\delta}{\partial\epsilon},$$

where the parentheses about the partial derivatives denote partial differentiation only in so far as ϵ enters ρ and ζ explicitly. Performing the differentiations and multiplying the results by ϵ so as to make the power of ϵ the same as that of the highest multiplicity of τ in its coefficient, we obtain

$$p = u_4 + K\tau u_3, \qquad q = v_4 + K\tau v_3,$$

$$u_4 = \frac{1}{2}(3 + \cos 2\tau)\epsilon^2 - \frac{1}{16}(51 + 12\cos 2\tau + \cos 4\tau)\epsilon^4 + - -,$$

$$v_4 = (\sin\tau)\epsilon + (\ )\epsilon^7 + - -,$$

$$K = -\frac{3}{2}\epsilon^2 + \frac{9}{2}\epsilon^4 + - -.$$

The criterion that the four sets of solutions that have been obtained constitute a fundamental set is that the determinant formed from these solutions and their first derivatives must be different from zero. This determinant is

$$\Delta = \begin{vmatrix} u_1 & u_2 & u_3 & u_4 + K\tau u_3 \\ \dot{u}_1 + i\alpha u_1 & \dot{u}_2 - i\alpha u_2 & \dot{u}_3 & \dot{u}_4 + K(\tau\dot{u}_3 + u_3) \\ iv_1 & -iv_2 & v_3 & v_4 + K\tau v_3 \\ i\dot{v}_1 - \alpha v_1 & -i\dot{v}_2 - \alpha v_2 & \dot{v}_3 & \dot{v}_4 + K(\tau\dot{v}_3 + v_3) \end{vmatrix} \tag{23}$$

Since the equations of variation do not contain τ explicitly, this determinant is a constant (4, §18). Its value may be obtained with the minimum of computation by putting $\tau = 0$. We thus obtain

$$\Delta = -2i\epsilon^2[1 + \epsilon^2(\text{a power series in } \epsilon^2)],$$

and this is different from zero for ϵ not zero but sufficiently small numerically. Hence the four solutions constitute a fundamental set, and the most general solutions of the equations of variation are

$$\begin{aligned} p &= K_1 e^{i\alpha\tau}u_1 + K_2 e^{-i\alpha\tau}u_2 + K_3u_3 + K_4(u_4 + K\tau u_3), \\ q &= i(K_1 e^{i\alpha\tau}v_1 - K_2 e^{-i\alpha\tau}v_2) + K_3v_3 + K_4(v_4 + K\tau v_3), \end{aligned} \tag{24}$$

where K_1, K_2, K_3, K_4 are arbitrary constants.

8. Notation

As we shall have frequent occasions in the sequel to use power series in odd or even powers of ϵ with sums of sines or cosines of odd or even multiples of τ in the coefficients, we shall adopt a notation* that will represent these series and denote their properties.

The letters C and S will denote the power series referred to above, C representing series with cosines in the coefficients and S with sines. Their

*This notation was first used by the author in his paper "Periodic orbits of the second genus near the straight line equilibrium points in the problem of three bodies" (1).

properties will be indicated by superscripts consisting usually of two parentheses. In the first parentheses will appear a digit followed by the letter o or e. The digit will represent the power of the lowest term in ϵ, and the parity of this digit will be the same as that of the parity of ϵ in the series, zero being taken as even. The letters o and e will signify that the multiples of τ in the sines or cosines are odd or even respectively.

The highest multiple of τ in the coefficient of ϵ^j is usually j but occasionally it exceeds j. When it is the same as j no second parenthesis in the superscript will appear. When it exceeds j a second parenthesis will be added containing a digit denoting the excess of the multiple over the power. Thus

$$C^{(0,e)} = \sum_{j=0}^{\infty} \sum_{k=0}^{j} (a_{2k}^{(2j)} \cos 2k\tau)\, \epsilon^{2j},$$

$$C^{(1,e)(1)} = \sum_{j=0}^{\infty} \sum_{k=0}^{j+1} (a_{2k}^{(2j+1)} \cos 2k\tau)\, \epsilon^{2j+1},$$

$$S^{(0,o)(1)} = \sum_{j=0}^{\infty} \sum_{k=0}^{j} (b_{2k+1}^{(2j)} \sin (2k+1)\tau)\, \epsilon^{2j}.$$

The symbols C and S with superscripts but without subscripts will denote *types* of series only. When a particular series of a type is to be noted, subscripts will be used.

Expressed in the above notation, the generating solutions and the u's and v's of the equations of variation are represented as follows:

$$\begin{aligned} \rho &= C^{(1,e)(1)}, & \zeta &= S^{(1,o)(1)}, \\ u_1 &= C^{(0,e)} + iS^{(0,e)}, & u_2 &= \bar{u}_1, \\ v_1 &= C^{(1,o)} + iS^{(1,o)}, & v_2 &= \bar{v}_1, \\ u_3 &= S_3^{(2,e)}, & v_3 &= C_3^{(1,o)}, \\ u_4 &= C_4^{(2,e)}, & v_4 &= S_4^{(1,o)}. \end{aligned}$$

9. The Highest-Multiple-Power Property

Certain pairs of series like u_3, u_4 have the property that the coefficient of the sine of the highest multiple of τ in the coefficient of any power of ϵ in one series is the same as, or differs only in sign from, the coefficient of the cosine of the highest multiple of τ in the coefficient of the same power of ϵ in the other series. This property will be called the Highest-Multiple-Power property and will be abbreviated to H.-M.-P. The word *same* or *opposite* will follow according as the coefficients in question are of the same or opposite signs, respectively. Thus

u_3, u_4, H.-M.-P. opposite
v_3, v_4, H.-M.-P. same.

10. Construction of the Periodic Solutions

We proceed to show that Equations (10) can be integrated as power series in λ and that γ and the constants of integration can be uniquely determined so that p and q will be periodic with the period T, a multiple of 2π, and will satisfy certain initial conditions.

Let us assume

$$p = \sum_{j=1}^{\infty} p_j \lambda^j, \quad q = \sum_{j=1}^{\infty} q_j \lambda^j, \quad \gamma = \sum_{j=1}^{\infty} \gamma_j \lambda^j, \tag{25}$$

and let these substitutions be made in Equations (10). The resulting equations will be cited as (10′). On equating the coefficients of the various powers of λ in Equations (10′) we obtain sets of differential equations from which the p_j and the q_j will be found. It will be shown that the γ_j and the arbitrary constants arising at each step can be so chosen as to satisfy the periodicity and certain initial conditions.

As the first genus orbits are symmetric, that is, $\dot{\rho}(0) = \zeta(0) = 0$, we shall confine our attention to the symmetric second genus orbits. Hence we put $\dot{p}(0) = q(0) = 0$ and when these conditions are imposed upon Equations (25) we have

$$\dot{p}_j(0) = q_j(0) = 0, \ (j = 1, 2, 3, - - -).$$

It will be found that, under the above initial conditions, one constant of integration will remain arbitrary at each step of the solutions for p_j and q_j. We may therefore impose an additional condition and we shall choose $\dot{p}(0) = 1$. Hence $p_1(0) = 1$, $p_j(0) = 0$, $j > 1$. There is no loss of generality here as p carries the factor λ in Equations (25). The parameter λ then becomes the scale factor of the second genus orbits.

Step 1. Coefficients of λ in Equations (10′)

On equating the coefficients of λ in Equations (10′) we obtain

$$\begin{aligned} \ddot{p}_1 + P_{11}p_1 + P_{12}q_1 &= P^{(1)} = \gamma_1 C^{(1,e)(1)} + C^{(1,e)(1)}, \\ \ddot{q}_1 + Q_{11}p_1 + Q_{12}q_1 &= Q^{(1)} = \gamma_1 S^{(0,o)(1)} + S^{(2,o)(1)}. \end{aligned} \tag{26}$$

The complementary functions of these equations are

$$\begin{aligned} p_1 &= k_1^{(1)} e^{i\alpha\tau} u_1 + k_2^{(1)} e^{-i\alpha\tau} u_2 + k_3^{(1)} u_3 + k_4^{(1)}(u_4 + K\tau u_3), \\ q_1 &= i(k_1^{(1)} e^{i\alpha\tau} v_1 - k_2^{(1)} e^{-i\alpha\tau} v_2) + k_3^{(1)} v_3 + k_4^{(1)}(v_4 + K\tau v_3), \end{aligned} \tag{27}$$

where $k_1^{(1)}, - - -, k_4^{(1)}$ are the arbitrary constants. By using the method of the variation of parameters to find the particular integrals we have

$$\begin{aligned} &\dot{k}_1^{(1)} e^{i\alpha\tau} u_1 + \dot{k}_2^{(1)} e^{-i\alpha\tau} v_2 + \dot{k}_3^{(1)} u_3 + \dot{k}_4^{(1)}(u_4 + K\tau u_3) = 0, \\ &\dot{k}_1^{(1)} e^{i\alpha\tau}(\dot{u}_1 + i\alpha u_1) + \dot{k}_2^{(1)} e^{-i\alpha\tau}(\dot{u}_2 - i\alpha u_2) \\ &\qquad + \dot{k}_3^{(1)} \dot{u}_3 + \dot{k}_4^{(1)}\{\dot{u}_4 + K(\tau \dot{u}_3 + u_3)\} = P^{(1)}, \\ &\dot{k}_1^{(1)} e^{i\alpha\tau} i v_1 - \dot{k}_2^{(1)} e^{-i\alpha\tau} i v_2 + \dot{k}_3^{(1)} v_3 + \dot{k}_4^{(1)}(v_4 + K\tau v_3) = 0, \\ &\dot{k}_1^{(1)} e^{i\alpha\tau}(i\dot{v}_1 - \alpha v_1) - \dot{k}_2^{(1)} e^{-i\alpha\tau}(i\dot{v}_2 + \alpha v_2) \\ &\qquad + \dot{k}_3^{(1)} \dot{v}_3 + \dot{k}_4^{(1)}\{\dot{v}_4 + K(\tau \dot{v}_3 + v_3)\} = Q^{(1)}. \end{aligned} \tag{28}$$

The determinant of $\dot{k}_1^{(1)}, - - -, \dot{k}_4^{(1)}$ is the same as in Equations (24) and therefore does not vanish for $\epsilon \neq 0$ but small numerically. Equations (28) are therefore solvable, the solutions being

$$\begin{aligned} \Delta\dot{k}_1^{(1)} &= - e^{-i\alpha\tau}(M_{12}P^{(1)} + M_{14}Q^{(1)}), \\ \Delta\dot{k}_2^{(1)} &= \quad e^{i\alpha\tau}(M_{22}P^{(1)} + M_{24}Q^{(1)}), \\ \Delta\dot{k}_3^{(1)} &= \quad - (M_{32}P^{(1)} + M_{34}Q^{(1)}), \\ \Delta\dot{k}_4^{(1)} &= \quad (M_{42}P^{(1)} + M_{44}Q^{(1)}), \end{aligned} \tag{29}$$

where $M_{jk}(j = 1, 2, 3, 4; k = 2, 4)$ are the co-factors of the elements in the determinant Δ, j referring to the column and k to the row. The forms of these co-factors in terms of the notation adopted, together with their properties, are given in Table I.

TABLE I

THE CO-FACTORS AND THEIR PROPERTIES

Co-factors	Type of series	Functions	H.-M.-P. properties
$M_{12} = \bar{M}_{22}$	$\epsilon^2(C_{12}^{(0,e)} + iS_{12}^{(0,e)})$		
$M_{14} = \bar{M}_{24}$	$i\epsilon^2(C_{14}^{(1,o)} + iS_{14}^{(1,o)})$		
M_{32}	$i(C_{32}^{(2,e)} + K\tau S_{42}^{(2,e)})$	$C_{32}^{(2,e)}$, $S_{42}^{(2,e)}$	Opposite
M_{42}	$iS_{42}^{(2,e)}$		
M_{34}	$i(S_{34}^{(1,o)} + K\tau C_{44}^{(1,o)})$	$S_{34}^{(1,o)}$, $C_{44}^{(1,o)}$	Same
M_{44}	$iC_{44}^{(1,o)}$		

On making the necessary substitutions in the first two of Equations (29), integrating and substituting the results in Equations (27) we obtain particular integrals for p_1, q_1 which are respectively of the same form as the right members of Equations (26). Non-periodic terms, however, will arise if α is an even integer. Those values of ϵ that will make α an even integer must therefore be excluded.

The integration of the last two of Equations (29) proceeds differently from the preceding integration. When the series involved are expressed in the notation employed,

$$\dot{k}_3^{(1)} = \frac{1}{\epsilon}\gamma_1 d_1^{(1)} + \epsilon d_2^{(1)} + \gamma_1 C_{32}^{(1,e)(3)} + C_{42}^{(1,e)(3)} + K\tau(\gamma_1 S_3^{(1,e)(3)} + S_4^{(1,e)(3)}),$$

$$\dot{k}_4^{(1)} = -\gamma_1 S_{32}^{(1,e)(3)} - S_{42}^{(1,e)(3)} - \gamma_1 S_3^{(1,e)(3)} - S_4^{(1,e)(3)},$$

where

$C_{32}^{(1,e)(3)}$, $S_{32}^{(1,e)(3)}$ have H.-M.-P. opposite,

$C_{42}^{(1,e)(3)}$, $S_{42}^{(1,e)(3)}$ have H.-M.-P. opposite,

and where the $d_i^{(1)}$ here and in the sequel denote power series in ϵ^2 with constant terms different from zero.

When the integrations are performed and the results for $k_3^{(1)}$ and $k_4^{(1)}$ are substituted in Equations (27), the non-periodic terms arising from the integration of $\gamma_1 S_3^{(1,e)(3)} + S_4^{(1,e)(3)}$ in $\dot{k}_3^{(1)}$, $\dot{k}_4^{(1)}$ cancel off and we obtain

$$\begin{aligned} p_1 &= \gamma_1 C^{(3,e)(1)} + C^{(3,e)(1)} + K_3^{(1)} u_3 + \left(KK_4^{(1)} + \frac{1}{\epsilon}\gamma_1 d_1^{(1)} + \epsilon d_2^{(1)}\right)\tau u_3, \\ q_1 &= \gamma_1 S^{(2,e)(1)} + S^{(2,e)(1)} + K_3^{(1)} v_3 + \left(KK_4^{(1)} + \frac{1}{\epsilon}\gamma_1 d_1^{(1)} + \epsilon d_2^{(1)}\right)\tau v_3. \end{aligned} \tag{30}$$

Because of the H.-M.-P. properties listed, the numbers in the second parentheses of the superscripts of C and S are 1 instead of 3.

From the periodicity conditions we must put

$$KK_4^{(1)} + \frac{1}{\epsilon}\gamma_1 d_1^{(1)} + \epsilon d_2^{(1)} = 0, \tag{31}$$

and the complete solutions at the first step become

$$\begin{aligned} p_1 &= K_1^{(1)}e^{i\alpha\tau}u_1 + K_2^{(1)}e^{-i\alpha\tau}u_2 + K_3^{(1)}u_3 + K_4^{(1)}u_4 + \gamma_1 C^{(1,e)(1)} + C^{(1,e)(1)}, \\ q_1 &= i(K_1^{(1)}e^{i\alpha\tau}v_1 - K_2^{(1)}e^{-i\alpha\tau}v_2) + K_3^{(1)}v_3 + K_4^{(1)}v_4 + \gamma_1 S^{(0,o)(1)} + S^{(2,o)(1)}, \end{aligned} \tag{32}$$

where $K_1^{(1)}, - - -, K_4^{(1)}$ are the constants of integration. The part of the particular integrals in Equations (30) obtained from the integration of $\dot{k}_3^{(1)}$ and $\dot{k}_4^{(1)}$ have been absorbed by the corresponding particular integrals arising from $\dot{k}_1^{(1)}$ and $\dot{k}_2^{(1)}$.

The period of the above solutions must be a multiple of $2\pi/\alpha$ and also of 2π. Hence α must be rational, i.e., $\alpha = N_1/N$, where N_1 and N are relatively prime integers. The period will then be

$$T = N_1\left(\frac{2\pi}{\alpha}\right) = N(2\pi). \tag{33}$$

From the initial conditions $\dot{p}_1(0) = 0$, or $q_1(0) = 0$, and $p_1(0) = 1$, it follows that

$$K_1^{(1)} = K_2^{(1)} = 1, \qquad K_3^{(1)} = 0.$$

The solutions, therefore, which satisfy the initial and periodicity conditions are

$$\begin{aligned} p_1 &= e^{i\alpha\tau}u_1 + e^{-i\alpha\tau}u_2 + K_4^{(1)}u_4 + \gamma_1 C^{(1,e)(1)} + C^{(1,e)(1)}, \\ q_1 &= i(e^{i\alpha\tau}v_1 - e^{-i\alpha\tau}v_2) + K_4^{(1)}v_4 + \gamma_1 S^{(0,o)} + S^{(2,o)(1)}, \end{aligned} \tag{34}$$

where $K_4^{(1)}$ and γ_1 must satisfy Equation (31).

Step 2. Terms in λ^2

It will be necessary to consider the terms in λ^2 where another relation between $K_4^{(1)}$ and γ_1 will arise which together with Equation (31) will uniquely determine these constants.

The differential equations at this step are

$$\begin{aligned} \ddot{p}_2 + P_{11}p_2 + P_{12}q_2 &= P^{(2)}, \\ \ddot{q}_2 + Q_{11}p_2 + Q_{12}q_2 &= Q^{(2)}, \end{aligned} \tag{35}$$

and the complementary functions are the same as Equations (27) with $k_1^{(2)}, - - -, k_4^{(2)}$ as the arbitrary constants. We shall require first only the terms in $e^{\pm i\alpha\tau}$ in the right members. So far as these terms are concerned,

$$\begin{aligned} P^{(2)} &= e^{i\alpha\tau}[\epsilon K_4^{(1)}(C^{(2,e)} + iS^{(2,e)}) + \gamma_1(C^{(0,e)} + iS^{(0,e)}) + C^{(2,e)} + iS^{(2,e)}] \\ &\quad + e^{-i\alpha\tau}[\quad \text{conjugate} \quad], \\ Q^{(2)} &= ie^{i\alpha\tau}[\epsilon K_4^{(1)}(C^{(1,o)} + iS^{(1,o)}) + \gamma_1(C^{(1,o)} + iS^{(1,o)}) + C^{(1,o)} + iS^{(1,o)}] \\ &\quad - ie^{-i\alpha\tau}[\quad \text{conjugate} \quad]. \end{aligned} \tag{36}$$

In the above and in the sequel the word "conjugate" in the parentheses designates the conjugate of the function in the parenthesis immediately preceding.

On varying the parameters $k_1^{(2)}$, $k_2^{(2)}$ we obtain

$$\begin{aligned} \Delta\dot{k}_1^{(2)} &= -e^{-i\alpha\tau}[M_{12}P^{(2)} + M_{14}Q^{(2)}], \\ \Delta\dot{k}_2^{(2)} &= e^{i\alpha\tau}[M_{22}P^{(2)} + M_{24}Q^{(2)}]. \end{aligned} \tag{37}$$

When the substitutions are made for the M's, P's, and Q's in Equations (37), constant terms will arise which must be annulled if the solutions at this step are to be periodic. This gives the second relation in $K_4^{(1)}$ and γ_1

$$\epsilon K_4^{(1)} d_1^{(2)} + \frac{1}{\epsilon^2}\gamma_1 d_2^{(2)} + d_3^{(2)} = 0, \tag{38}$$

which along with Equation (31) will give

$$K_4^{(1)} = \frac{1}{\epsilon} d_4^{(2)}, \qquad \gamma_1 = \epsilon^2 d_5^{(2)}. \tag{39}$$

On substituting Equations (39) in Equations (34) the solutions at the first step take the form

$$\begin{aligned} p_1 &= e^{i\alpha\tau}u_1 + e^{-i\alpha\tau}u_2 + C^{(1,e)(1)}, \\ q_1 &= e^{i\alpha\tau}iv_1 - e^{-i\alpha\tau}iv_2 + S^{(0,o)(2)}. \end{aligned} \tag{40}$$

When we make use of Equations (40) instead of Equations (34), the right members $P^{(2)}$, $Q^{(2)}$ take the form

$$\begin{aligned} P^{(2)} &= \epsilon e^{2i\alpha\tau}(C^{(0,e)} + iS^{(0,e)}) + \epsilon e^{-2i\alpha\tau} \text{ (conjugate)} \\ &\quad + \epsilon^2 e^{i\alpha\tau}(C^{(0,e)} + iS^{(0,e)}) + \epsilon^2 e^{-i\alpha\tau} \text{ (conjugate)} \\ &\quad + C^{(1,e)(1)}, \\ Q^{(2)} &= \epsilon e^{2i\alpha\tau}(iC^{(1,o)} + S^{(1,o)}) + \epsilon e^{-2i\alpha\tau} \text{ (conjugate)} \\ &\quad + e^{i\alpha\tau}(iC^{(1,o)} + S^{(1,o)}) + e^{-i\alpha\tau}\text{(conjugate)} \\ &\quad + S^{(2,o)(1)}. \end{aligned}$$

Varying the parameters $k_3^{(2)}$, $k_4^{(2)}$ we have equations similar to the last two Equations (29), if we replace the superscript (1)with (2). These two equations will give a relation between $K_4^{(2)}$ and γ_2 similar to Equation (31). When the integrations are completed and we impose the further condition $p_2(0) = 0$ we obtain

$$\begin{aligned} p_2 &= \sum_{j=1}^{2} (e^{ij\alpha\tau} U_{j1}^{(2)} + e^{-ij\alpha\tau} U_{j2}^{(2)}) + C^{(1,e)(1)}, \\ q_2 &= i \sum_{j=1}^{2} (e^{ij\alpha\tau} V_{j1}^{(2)} - e^{-ij\alpha\tau} V_{j2}^{(2)}) + S^{(2,o)(1)}, \end{aligned}$$

where $U_{j1}^{(k)}$ is similar to u_1, $V_{j1}^{(k)}$ to v_1 ; $U_{j1}^{(k)}$, $U_{j2}^{(k)}$ are conjugates and so also are $V_{j1}^{(k)}$, $V_{j2}^{(k)}$.

The integration for the higher terms in p and q may be carried on as far as desired. One relation $K_4^{(\rho)}$ and γ_j will be obtained at the step where $K_4^{(\rho)}$ arises and a second relation will be found at the next succeeding step. The general form will therefore be

$$\begin{aligned} p_n &= \sum_{j=1}^{n} (e^{ij\alpha\tau} U_{j1}^{(n)} + e^{-ij\alpha\tau} U_{j2}^{(n)}) + C^{(1,e)(1)}, \\ q_n &= i \sum_{j=1}^{n} (e^{ij\alpha\tau} V_{j1}^{(n)} - e^{-ij\alpha\tau} V_{j2}^{(n)}) + S^{(2,o)(1)}, \end{aligned}$$

and the final form for r and z is

$$r = \frac{c^2}{\mu}[1 + \epsilon(\rho + \sum_{n=1}^{\infty} p_n\lambda^n)],$$

$$z = \frac{c^2}{\mu}\epsilon(\zeta + \sum_{n=1}^{\infty} q_n\lambda^n).$$

The period in t of the first genus is

$$T_1 = \frac{c^3}{\mu^2}(1 + \delta)^{\frac{1}{2}}\, 2\pi$$

and of the second

$$T = N_1 T_1 (1 + \sum_{n=1}^{\infty} \gamma_n\lambda^n).$$

11. The Convergence of the Solutions

Only the formal construction of the solutions has been made and it now remains to consider their convergence. The usual method of establishing the convergence is by an existence proof based upon Poincaré's extension to Cauchy's theorem (5, Vol. 1, pp. 58-63). Such existence proofs are long and actually more difficult than the formal construction. An alternative method is to make use of MacMillan's theorem (3) where he showed that if the constants of integration can be determined so as to make the solutions of differential equations similar to those arising here *formally* periodic, then such solutions will converge for all finite values of the time, provided the scale factor, such as λ, is sufficiently small numerically.

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CONTRIBUTION À L'ÉTUDE DES SEMICARBAZIDES δ-SUBSTITUÉES[1]

II. SEMICARBAZONES DE QUELQUES ALDÉHYDES ET CÉTONES

PAR ROGER BARRÉ[2] ET LUCIEN PICHÉ[3]

Sommaire

La transformation semicarbazide⇄semicarbazone est une réaction réversible, qui donne lieu à un équilibre; théoriquement, du point de vue purement énergétique, la condensation d'une aldéhyde ou d'une cétone avec la semicarbazide simple ou substituée ne fournit jamais un rendement théorique de semicarbazone. Il est cependant démontré qu'on peut obtenir une réaction complète si on ajuste à leur optimum les conditions qui favorisent la condensation et surtout si on élimine la semicarbazone au fur et à mesure de sa formation.

Nous avons étudié plusieurs semicarbazides δ-substituées au point de vue de la solubilité relative de leurs semicarbazones; de tous ces dérivés, la *p*-nitrophényl-4-semicarbazide est celui qui présente les caractères les plus favorables à la précipitation des aldéhydes et des cétones. Son aptitude réactionnelle, sa stabilité, sa facilité relative de préparation et la faible solubilité de ses semicarbazones en font un réactif excellent des substances carbonylées; elle fournit un précipité quantitatif avec l'acétone, la benzaldéhyde, l'acétophénone, la *m*-nitrobenzaldéhyde, la vanilline, etc. Aussi la recommendons-nous comme réactif d'identification et de dosage des aldéhydes et des cétones.

Introduction

Pour l'identification et l'isolement des aldéhydes et des cétones, la semicarbazide compte au nombre des meilleurs réactifs; elle possède ce que Michael (8) a défini comme un "potentiel d'affinité élevé". Avec les sucres, elle conduit à des semicarbazones peu solubles et bien cristallisées, sans passer par l'intermédiaire de réactions antagonistes d'oxydo-réduction, comme cela se produit lors de la formation des osazones (7).

A cause du caractère essentiellement réversible de la réaction de condensation de la semicarbazide avec les aldéhydes et les cétones, la formation des semicarbazones est évidemment limitée, à chaque température et pour chaque composition du milieu réactionnel, par la réaction inverse d'hydrolyse. Depuis quelque temps, on a cherché à déterminer quels sont les facteurs qui fixent l'équilibre inévitablement atteint par la réaction en milieu homogène (3, 4); on s'est aussi appliqué à étudier les variations de vitesse de la condensation ou de la réaction inverse d'hydrolyse, en fonction de la nature du milieu (1, 5, 10).

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En système homogène, lorsque la solution est suffisamment diluée pour empêcher la précipitation de la semicarbazone, la réaction ne peut pas théoriquement conduire à un rendement quantitatif de semicarbazone; à chaque instant de la condensation, la vitesse est proportionnelle au produit des concentrations des deux substances à combiner et elle tend plus ou moins rapidement vers l'équilibre; il faut toutefois prévoir, en se basant sur les propriétés générales des équilibres chimiques, que la réaction sera complète si, ajustant à leur optimum les conditions qui accélèrent la condensation, on favorise en même temps l'élimination d'au moins un des produits de la réaction.

Cet objectif est en partie atteint si l'on opère en solution concentrée; le milieu se sature rapidement de semicarbazone relativement peu soluble, et l'élimine sous forme de précipité. On y arrive toutefois d'une façon plus efficace en utilisant la semicarbazide sous forme de combinaison à un radical organique de poids moléculaire élevé, rendant moins solubles les semicarbazones qui résultent de sa réaction avec les aldéhydes et les cétones. C'est en vue de ce résultat, déjà connu pour les hydrazones et d'ailleurs observé pour les semicarbazones elles-mêmes (6, 9, 11), que nous avons préparé plusieurs dérivés de la phényl-4-semicarbazide (2).

Partie expérimentale et résultats

L'influence relative des radicaux aromatiques sur la solubilité dans l'eau des semicarbazones substituées est exposée dans le Tableau I des solubilités respectives des semicarbazones fournies, par exemple, avec l'acétone et le glucose. Les semicarbazones du glucose figurent dans ce tableau parce que le principal objectif de notre travail était de déterminer quel dérivé de la semicarbazide se prête le mieux à des essais de précipitation quantitative de ce sucre.

L'examen de ces données révèle que la solubilité des semicarbazones ne dépend pas, rigoureusement de l'ordre de grandeur de leur poids moléculaire,

TABLEAU I

SOLUBILITÉ DANS L'EAU DES SEMICARBAZONES δ-SUBSTITUÉES DE L'ACÉTONE ET DU GLUCOSE

Semicarbazides	P.M.	Acétone-semicarbazone		Glucose-semicarbazone	
		P.M.	Solubilité, % (20° C.)	P.M.	Solubilité, % (20° C.)
Semicarbazide simple	75.1	115.1	6.22	237.2	2.25
Phényl-4-semicarbazide	151.1	191.2	0.09	313.2	7.57
p-Bromophényl-4-semicarbazide	230.0	270.1	0.02	392.1	0.64
p-Nitrophényl-4-semicarbazide	196.1	236.1	0.006	358.2	0.19
o, *p*-Dinitrophényl-4-semicarbazide	241.1	281.1	0.001	403.2	—
Benzyl-4-semicarbazide	165.1	205.1	0.20	327.2	11.50
Diphényl-4,4-semicarbazide	227.1	267.2	—	389.3	0.40
p-Xényl-4-semicarbazide	227.1	267.2	0.0002	389.3	0.05
p-Nitroxényl-4-semicarbazide	272.1	312.2	0.0001	434.2	—
Xanthyl-4-semicarbazide	257.1	297.2	0.002	419.3	0.03

mais bien plus de la nature du radical substituant qu'elles contiennent. Les dérivés les moins solubles sont fournis par les radicaux xényle (*p*-biphényle) et xanthyle, mais leurs semicarbazones cristallisent avec difficulté et se présentent sous forme de gelées qui retiennent toujours avec énergie une certaine quantité d'eau. Il est cependant possible de les isoler, pour fins d'identification et d'analyse, en les préparant dans l'alcool absolu et en distillant presque à sec après la condensation.

La présence du groupement nitré est nettement favorable à l'insolubilisation des semicarbazones et son influence se compare avantageusement à celle du groupement bromé ou à celle d'un second noyau benzénique. La *p*-nitrophényl-4-semicarbazide, en particulier, est caractérisée par une stabilité, par une rapidité et une uniformité d'action telles que de toutes les semicarbazides que nous ayons eu l'occasion de combiner avec les aldéhydes et les cétones, elle est celle qui se prête le mieux à leur identification par précipitation quantitative. Voici les points de fusion des semicarbazones que ce réactif nous a fournis avec les aldéhydes et les cétones suivantes (points de fusion instantanée; méthode Maquenne, indices non corrigés):—

p-Nitrophényl-4-semicarbazones	P.F., ° C.
Acétone	264
Benzaldéhyde	235–236
m-Nitrobenzaldéhyde	276
Vanilline	261
Acide glyoxylique	249
Acide pyruvique	261
Glucose	192–193

Ces semicarbazones peuvent servir au dosage des substances carbonylées correspondantes; la précipitation est rapide et totale et elle est pratiquement indépendante de la nature du milieu où elle se produit.

Ces caractères, comparés aux propriétés équivalentes des nombreux dérivés hydraziniques ou semicarbazidiques qui sont utilisés comme réactifs de dosage des aldéhydes ou des cétones, nous laissent la conviction que la *p*-nitrophényl-4-semicarbazide est, pour cet usage, le réactif le plus efficace et le mieux adapté.

TABLEAU II

POINTS DE FUSION (° C.) DES DÉRIVÉS DE QUELQUES SEMICARBAZIDES δ-SUBSTITUÉES

Semicarbazides	Base libre	Chlorhydrate	Acétone-semicarbazone	Glucose-semicarbazone
Phényl-4-semicarbazide	123	215	160–1	*161*
p-Bromophényl-4-semicarbazide	254	218–21	174	*168*
p-Nitrophényl-4-semicarbazide	*191*	*215*	*264*	*192–3*
o, *p*-Dinitrophényl-4-semicarbazide	*178*	—	*248*	—
Benzyl-4-semicarbazide	111	224	113	*115*
p-Nitrobenzyl-4-semicarbazide	*164*	*195–7*	*162*	—
Diphényl-4,4-semicarbazide	154	218–20	119	164–6
p-Xényl-4-semicarbazide	258	*308*	*228*	*194*
p-Nitroxényl-4-semicarbazide	*178*	*219*	*261*	*172*
Xanthyl-4-semicarbazide	174	—	265	*183*

Pour servir d'indices d'identification, nous donnons dans le Tableau II, les points de fusion des semicarbazones de l'acétone et du glucose que nous avons obtenus avec quelques dérivés de la semicarbazide; les indices en italiques correspondent à des déterminations originales.

Conclusions

En dépit du caractère essentiellement réversible de la réaction de condensation des dérivés semicarbazidiques avec les aldéhydes et les cétones, il est possible d'obtenir un rendement quantitatif de semicarbazones, grâce à la faible solubilité de quelques-unes de celles-ci. Les caractères tout à fait particuliers de la *p*-nitrophényl-4-semicarbazide, sa stabilité, son uniformité d'action et l'insolubilité de ses semicarbazones nous la font recommander comme réactif d'identification et de dosage des substances carbonylées.

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THE IDENTIFICATION OF BIOS V AS VITAMIN B_1 AND OF A CONSTITUENT OF BIOS VII SOLUTION AS VITAMIN B_6; THEIR EFFECT UPON THE REPRODUCTION OF *SACCHAROMYCES HANSENIASPORA VALBYENSIS*, YEAST 2335, AND *SACCHAROMYCES GALACTOSUS*[1]

By Cosmo Marchant[2]

Abstract

Experiments described in this paper show that both *Saccharomyces hanseniaspora valbyensis* and Yeast 2335 require the presence of crude Bios IIA, purified Bios IIB, vitamin B_6 (in place of Bios VII solution), and vitamin B_1 (in place of Bios V solution) in addition to *meso*-inositol in the culture medium to ensure a good crop; that *Saccharomyces galactosus* requires crude Bios IIA, purified Bios IIB, Bios VII solution (not vitamin B_6), and vitamin B_1 (in place of Bios V solution). They further show that the intermediates used in the synthesis of vitamin B_1 when added to a medium containing no vitamin B_1 increase the crop of Yeast 2335 and of *S. galactosus*, but not of *S. hanseniaspora valbyensis*.

Introduction

The constituents of Bios which give good crops of *Saccharomyces cerevisiae* (1, 6, 10) are not sufficient to ensure good crops of certain other species of yeast. Miss Farrell (3), for instance, found that *Saccharomyces hanseniaspora valbyensis* needed not only *meso*-inositol, crude Bios IIA,* and crude Bios IIB,* but also some other constituent of wort, tomato juice, or yeast water. To this unknown she gave the name Bios V. Miss Elder (2) showed that, in addition to *meso*-inositol, purified Bios IIA,* purified Bios IIB* and Bios V, *S. hanseniaspora valbyensis* needs two others (not required by *S. cerevisiae*); one of these (Bios VII) is contained in the crude Bios IIB solution and the other (Bios VIII) in the crude Bios IIA solution. Maconachie (9) found that Yeast 2335 of the American Type Culture Collection required *meso*-inositol and Bios II (crude Bios IIA and crude Bios IIB). Miss Farrell and Miss Elder found that in media made up with (*a*) "Bios V Reagent",** (*b*) inositol, crude Bios IIA, and crude Bios IIB, and (*c*) tomato juice, *S. cerevisiae* gave crops of the same size, but *Saccharomyces galactosus* and

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* *The term purified Bios IIA signifies that the crude Bios IIA solution has been freed of traces of Bios VIII as well as other impurities, while the term crude Bios IIA indicates that the solution contains both Bios VIII and purified Bios IIA. Similarly, crude Bios IIB solution contains both purified Bios IIB and Bios VII, while purified Bios IIB solution contains no Bios VII.*

** *"Bios Reagent" is obtained by adding tannin and basic lead acetate to tomato juice, filtering off the precipitate, heating the filtrate with excess calcium hydroxide at 100° C. for 40 min. to destroy Bios V, filtering, and removing excess lime by passing carbon dioxide into the hot solution. It thus contains all the constituents of Bios except Bios V.*

Yeast 2335 gave much smaller crops in (*a*) and (*b*) than in (*c*). Miss Elder also found that the addition of Bios V solution (in an amount that greatly increases the crop of *S. hanseniaspora valbyensis*) to media containing "Bios V Reagent" did not increase the crops of *S. galactosus* and Yeast 2335. She therefore drew the conclusion (later shown to be unjustified) that these two yeasts required at least one other unknown constituent which may or may not be the same for each yeast.

This paper describes the results of a study of Bios V, Bios VII, and Bios VIII and their effect upon the reproduction of *S. hanseniaspora valbyensis*, Yeast 2335, and *S. galactosus*.

Experimental

The Yeasts

Saccharomyces hanseniaspora valbyensis (A.T.C.C. 2108)

This culture was obtained from the A.T.C.C. in 1930. During the course of these experiments a strain (*N*) of this yeast was isolated which, with a fixed amount of Bios, gave two-thirds the crop given by a strain (*H*) obtained from the Centralbureau voor Schimmelcultures in Delft in 1938. This Strain *H* was derived from the original A.T.C.C. culture, since in 1936 a culture of *S. hanseniaspora valbyensis* had been sent to Delft from Toronto. The only other way in which these strains differed was in the rates of reproduction: For the first 12 hr. the rates were about the same, but that of Strain *N* fell off after this time; Strain *H* attained its maximum crop between 24 and 28 hr., Strain *N* between 28 and 32 hr., and the maximum crop of *H* was higher than that of *N*. No difference was observed in cell size or the appearance of colonies and, so far, no difference in the constituents of Bios required by each strain has been observed.

Yeast 2335

A culture of this yeast was purchased from the A.T.C.C. in May 1936. Miss Elder (2) found that when this yeast was plated out, two types of colonies developed in the ratio of about one smooth colony with radial furrows to eight fuzzy colonies; yeast from either type of colony on subsequent plating out remained true to type. For the experiments described below, Miss Elder's race F (Fuzzy) was used.

Saccharomyces galactosus

This culture was kindly supplied in 1932 by Dr. A. M. Wynne, of the Department of Biochemistry, University of Toronto, who had obtained it from the Labatorium voor Microbiologie der Technische Hoogeschool at Delft.

Procedure

The yeasts for seeding were cultivated by rocking at 25° C. in rocker tubes containing 10 cc. of tomato juice culture medium and transferring a loopful every 24 hr. to a fresh 10 cc. of medium (3, 9).

The method of Bios assay was the same as that described by Miss Elder (2).

Experiments with Bios V

Identification of Bios V as Vitamin B_1 (7)

On February 8, 1937, Dr. C. N. Frey of the Fleischmann Laboratories notified us that vitamin B_1 greatly accelerated the rate of fermentation of yeast (11). This substance (Merck's Betabion—vitamin B_1 hydrochloride) was at once tried, to ascertain whether it would replace any of the unknown constituents of the culture medium for *S. hanseniaspora valbyensis*.

The solutions used in all the experiments were prepared as follows. The inositol solutions were made from Eastman's Ash-free *meso*-inositol; the Bios V (acetone-purified) and "Bios V Reagent" were prepared by Miss Elder (2); the tomato juice was first freed of pulp, then neutralized (litmus) with sodium hydroxide and diluted to the required strength; the vitamin B_1 solutions were made from Merck's Betabion (thiamine hydrochloride) by dissolving in water, making slightly acid to litmus with acetic acid, and diluting to the desired strength; the crude Bios IIB, purified Bios IIB, and Bios VII solution were prepared as described on page 27; and the crude Bios IIA was prepared according to Miss Sanderson's recipe (5). The "concentrations" are cubic centimetres of tomato juice (t.j.) from which the solutions were formed, except where otherwise stated or where a particular chemical has been used. To every assay medium one constituent of Bios was added in a small amount so that it would be used up first and thus limit the size of the crop.

In Table I the crop-limiting constituent is either Bios VII solution or the Bios VII in the crude Bios IIB solution, when the latter is substituted for the purified Bios IIB and the Bios VII. It is obvious that for the purpose of this experiment it does not matter which constituent is present in the least (physiological) amount. The results (Table I) show that when vitamin B_1 replaced Bios V solution the crop was much larger than when the medium contained neither Bios V nor vitamin B_1 and was as large as that obtained when the medium contained Bios V; that when it was substituted for any of the other constituents, the crops were no larger than those obtained when any of them was omitted.

Subsequent experiments showed that vitamin B_1 can replace Miss Elder's "unknown constituent" for Yeast 2335 and *S. galactosus*.

Miss Elder (2) found that the addition of Bios V to media containing tomato juice did not increase the crop of *S. hanseniaspora valbyensis*, while the addition of "Bios V Reagent" did. In this respect also, vitamin B_1 is the same as Bios V. It follows that the amount of vitamin B_1 in the tomato juice is measured by the crop of *S. hanseniaspora valbyensis* only if the assay medium is made up with excess of "Bios V Reagent". On the other hand, with Yeast 2335 and *S. galactosus*, when a large quantity of vitamin B_1 (or Bios V) was added to media containing tomato juice the crops were greatly increased. As the addition of "Bios V Reagent", or inositol, crude Bios IIA, and crude Bios IIB, to a medium containing tomato juice did not increase

the crop of either yeast, the constituent in the least (physiological) amount for these two yeasts is vitamin B_1. With this in mind, measurements were made to determine the concentration of vitamin B_1 in tomato juice. The results obtained with *S. hanseniaspora valbyensis* are given in Table II. The measurements with Yeast 2335 and *S. galactosus* were made with the concentrations of the constituents outlined below, and also with twice the concentrations of dextrose, salts, and "Bios V Reagent" shown in Table II.

TABLE I

THE EFFECT ON THE GROWTH OF *S. hanseniaspora valbyensis** OF REPLACING BIOS V, VII, AND VIII BY VITAMIN B_1

Each cubic centimetre of assay medium contained 50 mg. of dextrose, 2.1 mg. of potassium dihydrogen phosphate, 4.2 mg. of ammonium nitrate, 1.0 mg. of magnesium sulphate heptahydrate, 0.35 mg. of calcium chloride hexahydrate, and 0.02 mg. of inositol, together with the ingredients§ given below.

Crude Bios IIA from cc. t.j.	Crude Bios IIB from cc. t.j.	Purified Bios IIB from cc. t.j.	Bios VII from cc. t.j.	Bios V from cc. t.j.	Vitamin B_1 γ	Count**
0.1	0.033	0	0	0	0	30
0.1	0.033	0	0	0.0225	0	250
0.1	0.033	0	0	0	0.33	246
0	0.033	0	0	0.0225	0	187
0	0.033	0	0	0.0225	0.33	180
0.1	0	0.05	0.05	0.0225	0	260
0.1	0	0.05	0	0.0225	0	30
0.1	0	0.05	0	0.0225	0.33	40
0.1	0	0	0.05	0.0225	0	20
0.1	0	0	0.05	0.0225	0.33	30

* *The measurements recorded in Tables I, II, and IV were carried out before the culture of S. hanseniaspora valbyensis had been shown to be a mixture of at least two strains. This does not invalidate the results, however, since all the measurements in any one table were made at the same time with the same yeast suspension.*

§ *These solutions contain none, or only traces, of the other constituents of Bios, except; (a) the crude Bios IIB, which contains purified Bios IIB and Bios VII; (b) crude Bios IIA, which contains purified Bios IIA and Bios VIII; and (c) "Bios V Reagent", which contains all constituents except Bios V.*

** *A count (C) of 1.0 = 250,000 cells per cc.*

It was soon seen that the amounts of vitamin B_1, tomato juice, and Bios V solution required to secure a given crop of Yeast 2335 are proportional to those required by *S. hanseniaspora valbyensis*, but from 67 to 87 times greater, and, as with *S. hanseniaspora valbyensis*, doubling the amounts of either Bios V solution or vitamin B_1 doubles the crop of Yeast 2335. To give a crop of about C = 252 the quantity of vitamin B_1 required by *S. galactosus* is about 31 times that required by *S. hanseniaspora valbyensis;* in contrast to what occurs with the other yeasts, doubling the amount multiplies the crop by 1.7. According to the experiments with *S. hanseniaspora valbyensis*, 1 cc. of tomato juice contains 0.57 γ of vitamin B_1; according to those with Yeast 2335, 0.62 γ; and those with *S. galactosus*, 0.54γ. The tomato juice

TABLE II

DETERMINATION OF THE CONCENTRATION OF VITAMIN B_1 IN TOMATO JUICE WITH *S. hanseniaspora valbyensis**

Each cubic centimetre of assay medium contained dextrose, salts, and "Bios V Reagent" from 0.09 cc. of tomato juice together with the ingredients given below

Tomato juice, cc.	Vitamin B_1, γ	Bios V from cc. t.j.	Vitamin B_1§, γ	Count
0	0	0	0	20
0.0015	0	0	0	320
0.0020	0	0	0	460
0	0.00062	0	0	238
0	0.00087	0	0	306**
0	0.00093	0	0	360
0	0.00124	0	0	527
0	0	0.0103	0	221
0	0	0.0145	0	320
0	0	0.0154	0	380
0	0	0.0206	0	440
0	0	0	0.00124	20

* *See footnote Table I.*

§ *Ten cc. of a solution containing 0.0062γ of vitamin B_1 per cc. was heated for 30 min. at 100° C. with 0.5 gm. of calcium hydroxide, filtered, and the excess lime removed by passing carbon dioxide into the hot solution. Two cc. of this solution was added to the assay medium.*

** *In identical assay media, Strain N gives a count of 240 and Strain H one of 360.*

used in the experiments was from a small tin of Libby's (Libby McNeill and Libby of Canada Ltd.) tomato juice. A later shipment of No. 10 tins of Libby's contained about 0.3γ vitamin B_1 per cc.; Silver Ribbon Brand (Baxter Canning Co.) tomato juice contained about 0.4γ per cc.

Since, to give about the same crop, Yeast 2335 and *S. galactosus* require much more vitamin B_1 than *S. hanseniaspora valbyensis*, it is easy to understand why Miss Elder failed to get any increase in the crops of these two yeasts, for she used the same amounts of Bios V as in her experiments with *S. hanseniaspora valbyensis*.

Both vitamin B_1 and Bios V occur in fruit juices, wort, and yeast water (3); the behaviour of the former with reagents, particularly the destruction of its activity on heating with lime water (Table II), is the same as that of Bios V; so there need be no hesitation in concluding that the active constituent of Bios V solution and that required by Yeast 2335 and *S. galactosus* is vitamin B_1.

THE EFFECT OF REPLACING VITAMIN B_1 BY "AMINOPYRIMIDINE" AND "THIAZOLE" (8)

In 1938, Dr. C. N. Frey *et al.* (12) reported that, with certain races of yeast, vitamin B_1 could be replaced by the intermediates used in its synthesis, viz.: 2-methyl-5-ethoxymethyl-6-aminopyrimidine and 4-methyl-5-β-hydroxyethylthiazole. A few milligrams of the "aminopyrimidine" was very kindly sent to us by Dr. Frey; for the "thiazole" we are indebted to Merck and Company Inc.

It was found that the "aminopyrimidine" and the "thiazole" when added singly or together to the assay medium produce little or no increase in the crop of either strain of *S. hanseniaspora valbyensis*. In other words, with *S. hanseniaspora valbyensis* vitamin B_1 cannot be replaced by its intermediates.

With Yeast 2335, addition of the "aminopyrimidine" alone has little effect on the crop; addition of 0.005γ of "thiazole" increases it, but it is not much further increased by adding 20 times that amount. Addition of "aminopyrimidine" to media containing "thiazole" increases the crop, but the addition of more and more of both does not increase it above C = 320 whether the assay medium contains "Bios V Reagent" from 0.2 cc. or from 0.3 cc. of tomato juice.

The crops obtained in media containing mixtures of the intermediates or "thiazole" alone are greater (at the lower concentrations) and smaller (at the higher concentrations) than those that would be obtained from the amount of vitamin B_1 that could be formed from them according to the equation:

$C_8H_{13}N_3O$	+	$C_6H_{10}NSO$	=	$C_{12}H_{18}N_4SOCl_2$
167.1γ		144.1γ		337.1γ
"Aminopyrimidine"		"Thiazole"		Vitamin B_1 hydrochloride

For, 0.012γ of vitamin B_1 (= 0.006γ of "aminopyrimidine" + 0.005γ of "thiazole", for which C = 110) would give a crop of C = 54, and 0.12γ of vitamin B_1 (= 0.06γ of "aminopyrimidine" + 0.05γ of "thiazole"; C = 320) would yield one of C = 540.

With *S. galactosus* (Table III), "thiazole" by itself has little or no effect on the crop (cf. Yeast 2335); 0.005γ of "aminopyrimidine" gives one of C = 178, while even 20 times that amount of "aminopyrimidine" gives a count only slightly higher.

TABLE III

THE EFFECT OF REPLACING VITAMIN B_1 WITH "AMINOPYRIMIDINE" AND "THIAZOLE"

Each cubic centimetre of assay medium contained dextrose and salts (twice the concentrations shown in Table I), inositol* and crude Bios IIA* from 0.2 cc. of tomato juice, and crude Bios IIB from 0.118 cc. of tomato juice, together with the ingredients given below

"Amino-pyrimidine" γ	"Thiazole" γ	Count	"Amino-pyrimidine" γ	"Thiazole" γ	Count
0	0	88	0	0.1	97
0.005	0	178	0.005	0.005	200
0.01	0	212	0.01	0.01	262
0.05	0	239	0.01	0.1	294
0.1	0	221	0.1	0.01	433
0	0.005	105	0.05	0.05	735
0	0.01	88	0.1	0.1	714
0	0.05	106			

* *For convenience, the charcoal filtrate (p. 27), which contains both meso-inositol and crude Bios IIA, was used in making up the assay media.*

Mixtures of the "intermediates" in amounts that are approximately equivalent to a given amount of vitamin B_1 give about the same crop as the vitamin B_1 when they replace it in the culture medium for *S. galactosus*. For example, 0.02γ of vitamin B_1, which is equivalent to 0.01γ of "aminopyrimidine" + 0.0084γ of "thiazole", gives a count of 252, while 0.01γ of "aminopyrimidine" + 0.01γ of "thiazole" gives a count of 262.

Experiments with Bios VII

The Preparation of Bios VII Solution

As the Bios IIB solution from sugar-refinery charcoal (7) contained no Bios VII,* that substance could not be used as a raw material. In order to obtain a sufficient quantity of Bios VII solution, 72 kg. of tomato juice (Silver Ribbon Brand, Baxter Canning Company) were worked up according to Miss Sanderson's recipe (5) in 14.4 kg. batches. And the crude Bios IIB was obtained by adsorption on charcoal after the method of Sparling (10). The 54 cc. of crude Bios IIB thus prepared contained 16% of the Bios VII present in the tomato juice from which it was obtained.

The Fractionation of Crude Bios IIB into Purified Bios IIB and Bios VII

To 1 cc. (0.93 gm. of non-volatile matter) of a neutral crude Bios IIB solution, 0.45 cc. of 10% sodium carbonate solution and 0.5 cc. of 25% mercuric acetate solution were added alternately four times. The volume was then brought to 34 cc. with 95% ethyl alcohol. After standing three hours, the precipitate (I) was filtered off. The filtrate was evaporated *in vacuo* to a volume of 1 cc. and the whole process repeated; but this time a total of 3.6 cc. of 10% sodium carbonate solution and 4.4 cc. of the mercury solution was used, then made up to 60 cc. with 95% alcohol. This precipitate (II) was filtered off after three hours. The mercury was removed as sulphide from all three fractions, viz.; the first and second precipitates and the final filtrate (the precipitates were suspended in dilute acetic acid solution for this operation). The resulting solutions were all brought to the same concentration, viz.; 1 cc. of solution from 0.33 cc. of tomato juice. The data regarding the crops of *S. hanseniaspora valbyensis* obtained with these solutions are given in Table IV.

The results show that the crops obtained in media containing either (*a*) the first precipitate, second precipitate, and final filtrate, or (*b*) the second precipitate and final filtrate, were almost as large as the crop obtained in a medium containing that amount of crude IIB from which the solutions had been obtained. In media containing any one by itself or any other combination of the solutions, the crop was small. Thus crude Bios IIB can be fractionated into two constituents (second precipitate and final filtrate), neither of which by itself gives any appreciable crop of *S. hanseniaspora valbyensis*,

* *Two relatively impure preparations of IIB from sugar-refinery charcoal were tried with S. hanseniaspora valbyensis. Neither replaced crude IIB, nor was either a poison; both could replace purified Bios IIB.*

TABLE IV

THE EFFECT OF THE FRACTIONS FROM CRUDE BIOS IIB ON THE REPRODUCTION OF *S. hanseniaspora valbyensis**

Each cubic centimetre of assay medium contained dextrose and salts, 0.02 mg. of inositol, crude Bios IIA from 0.01 cc. of tomato juice, and Bios V from 0.0225 cc. of tomato juice, together with the ingredients given below

Crude Bios IIB from cc. t.j.	Ppt. I from cc. t.j.	Ppt. II from cc. t.j.	Filtrate from cc. t.j.	Count
0	0	0	0	12
0.033	0	0	0	210
0	0.033	0.033	0.033	180
0	0.033	0.033	0	20
0	0	0.033	0.033	160
0	0.033	0	0.033	51
0	0.033	0	0	5
0	0	0.033	0	8
0	0	0	0.033	17

* *See footnote Table I.*

while both together give a large crop. The one that is not precipitated by mercury carbonate is Bios VII; the other is purified Bios IIB.

The remainder of the crude Bios IIB solution was treated in a similar manner with mercury carbonate. The final filtrate (after removing mercury as the sulphide) contained about 30% of the Bios VII present in the 54 cc. of crude IIB solution, i.e., about 5% of that in the 72 kg. of tomato juice. It was almost free of purified Bios IIB. The amount of non-volatile matter per unit of Bios VII* was 0.86 mg.; most of this was sodium acetate.

Removal of Sodium from the Bios VII Solution

The greater part of the sodium was removed by adding 31.2 cc. of 18.4 *N* sulphuric acid, evaporating *in vacuo* to remove the acetic acid, and extracting the dry residue with 680 cc. of 95% ethyl alcohol; this left most of the sodium sulphate behind. The alcoholic solution was evaporated *in vacuo* to dryness, and the residue was stirred with water; an appreciable amount remained undissolved. The aqueous solution was evaporated *in vacuo* to dryness, dissolved again in water, and a small amount of sulphate was removed as barium sulphate. After filtering, the solution contained 96% of the Bios VII present before the removal of sodium and only 17% of the non-volatile matter; quality, 0.15 mg./unit.

Further Purification with Alcohol

The solution of Bios VII so obtained was evaporated to dryness *in vacuo* and stirred with 600 cc. of 95% ethyl alcohol. A considerable amount of material was left undissolved. The alcoholic solution was evaporated to dryness; nearly all the residue dissolved in water. The aqueous solution was

* *Defined as that amount which, when all the other constituents are present in excess in the assay medium, gives a crop of C = 300 to 350 with S. hanseniaspora valbyensis N.*

evaporated to dryness and the residue dissolved again in water. After these operations there remained 98% of the Bios VII, whereas the amount of non-volatile matter was reduced by 28%; quality, 0.11 mg./unit.

The Identification of a Constituent of Bios VII Solution as Vitamin B_6 (8)

In August, 1938, shortly after a note (4) appeared reporting the isolation of pure crystals of the hydrochloride of vitamin B_6, Merck and Co. Inc. very kindly sent a milligram of this compound to us.

It was immediately found that both *S. hanseniaspora valbyensis N* and *H* gave a large crop in a medium containing inositol, crude IIA, vitamin B_1, and purified Bios IIB to which had been added vitamin B_6 (1.0γ per cc.) in place of Bios VII; but when vitamin B_6 was tried in place of Bios IIB the crop was small.

As in the case of vitamin B_1, experiments were carried out to determine the amounts of vitamin B_6 and Bios VII solution required to secure a given

TABLE V

DETERMINATION OF THE CONCENTRATION OF VITAMIN B_6 IN TOMATO JUICE WITH *S. hanseniaspora valbyensis*

Each cubic centimetre of assay medium contained dextrose, salts, inositol from 0.2 cc. of tomato juice, crude Bios IIA from 0.2 cc. of tomato juice, and 0.154 γ of vitamin B_1, together with the ingredients given below

Purified Bios IIB§, γ	Tomato juice, cc.	Bios VII*, cc. t.j.	Vitamin B_6, γ	Count	
				N	*H*
1.39	0	0	0	68	68
1.39	0.0013	0	0	—	180
1.39	0.0025	0	0	200	300
1.39	0.005	0	0	330	500
1.39	0.01	0	0	510	—
1.39	0	0.025	0	—	174
1.39	0	0.05	0	200	280
1.39	0	0.1	0	310	470
1.39	0	0.2	0	460	—
1.39	0	0	0.0005	—	170
1.39	0	0	0.001	190	238
1.39	0	0	0.00125	—	260
1.39	0	0	0.002	290	408
1.39	0	0	0.0025	340	—
1.39	0	0	0.004	430	—
0	0.0025	0	0	—	309
0	0.005	0	0	357	—
0	0	0.05	0	—	42
0	0	0.1	0	68	—
0	0	0	0.00125	—	25
0	0	0	0.0025	25	—

§ *This was prepared from sugar-refinery charcoal by extracting the charcoal with ethyl alcohol and ammonia, evaporating to dryness, taking up in water, and again evaporating to dryness. The residue was further purified by heating with methyl alcohol and hydrochloric acid and extracting with chloroform; this operation was repeated using ethyl alcohol. The concentration is expressed as the weight of non-volatile matter per cubic centimetre.*

* *This preparation contained 4.7% of the Bios VII present in the tomato juice from which it was prepared; therefore the preparation from 0.1 cc. of tomato juice should contain the Bios VII of 0.0047 cc. of tomato juice; this is so, for, in this table, 0.005 cc. of tomato juice was used.*

crop and to determine the concentration of vitamin B_6 in tomato juice. From the results of Table V it is seen that, with both *S. hanseniaspora valbyensis N* and *H*, vitamin B_6 can replace Bios VII solution; that, in contrast to what occurs with vitamin B_1 or Bios V, doubling the quantities of tomato juice, Bios VII solution, or vitamin B_6, multiplies the crop by about only 1.6; and that 1 cc. of tomato juice or Bios VII solution from 20 cc. of tomato juice can replace 0.4 to 0.5γ of vitamin B_6 whether the crop of *S. hanseniaspora valbyensis N* or *H* be measured.

While this manuscript was in preparation, a note (13) from Frey *et al.* appeared announcing that vitamin B_6 could replace vitamin B_1 in the culture medium for certain of his yeasts. Consequently, measurements were made to ascertain whether this would hold for *S. hanseniaspora valbyensis*. In contrast to Frey's findings with his yeasts, vitamin B_6 cannot replace vitamin B_1 in the culture medium for *S. hanseniaspora valbyensis H*.

TABLE VI

DATA SHOWING VITAMIN B_6 CANNOT REPLACE VITAMIN B_1 IN THE CULTURE MEDIUM FOR *S. hanseniaspora valbyensis*

Each cubic centimetre of assay medium contained dextrose, salts, 0.04 mg. of inositol, crude Bios IIA from 0.1 cc. of tomato juice, and crude Bios IIB from 0.033 cc. of tomato juice, together with the ingredients given below

Vitamin B_1	Vitamin B_6	Count
0	0	50
0.00087	0	350
0	0.00125	60

Measurements made with Yeast 2335 showed that small crops of this yeast were obtained in a medium containing inositol, crude Bios IIA, and vitamin B_1, but neither purified Bios IIB nor Bios VII solution; a somewhat larger crop when either purified Bios IIB or Bios VII solution was added; and a much larger crop when both were added together. Moreover, vitamin B_6 can replace Bios VII solution. Doubling the amounts of tomato juice, Bios VII, or vitamin B_6 multiplies the crop by about 1.4 (contrast vitamin B_1 or Bios V); to secure a crop of C = 300, about four times the quantity of Bios VII solution or vitamin B_6 required by *S. hanseniaspora valbyensis N* is necessary. The results also confirmed those of Table V, namely, that 1 cc. of tomato juice or Bios VII solution from 20 cc. of tomato juice contains 0.4 to 0.5γ of vitamin B_6.

Thus the active principle for *S. hanseniaspora valbyensis* and Yeast 2335 in the Bios VII solution is vitamin B_6.

On the other hand, small crops of *S. galactosus* were obtained in media containing (besides inositol, crude Bios IIA, and vitamin B_1) either Bios VII

solution or purified Bios IIB and vitamin B_6, but when the medium contained inositol, crude Bios IIA, vitamin B_1, purified Bios IIB, and Bios VII solution the crop was large.

Therefore, *S. galactosus*, like the others, requires purified Bios IIB and Bios VII solution, but as the latter cannot be replaced by vitamin B_6, it must be assumed either that the Bios VII solution contains two active constituents, viz., vitamin B_6 and the constituent required by *S. galactosus*, or that there is some chemical other than vitamin B_6 that behaves with *S. hanseniaspora valbyensis* and Yeast 2335 just as vitamin B_6 does.

Experiments with Bios VIII

When it was discovered that, with *S. cerevisiae*, Bios IIA can be replaced by β-alanine alone or *l*-leucine plus β-alanine (6), the same replacements were tried with *S. hanseniaspora valbyensis*, Yeast 2335, and *S. galactosus*.

The results showed that β-alanine (about 200 times the amount used with *S. cerevisiae*) alone or β-alanine plus *l*-leucine cannot replace crude Bios IIA in the culture medium for *S. hanseniaspora valbyensis*, but they did not show whether β-alanine and *l*-leucine are constituents of Bios for this yeast in addition to the unknown constituent (Bios VIII) in the crude Bios IIA.

The similar measurements made with Yeast 2335 and *S. galactosus* showed that β-alanine plus *l*-leucine cannot take the place of crude Bios IIA with either yeast, but did not show whether β-alanine and *l*-leucine are necessary to ensure good crops of these yeasts.

Thus, the production of good crops of the three yeasts requires the presence, in the culture medium, of some unknown constituent (perhaps the same for all) called provisionally Bios VIII, which is present in the crude Bios IIA solution.

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